


## RESEARCH ARTICLE

# A pilot study to explore the effect of udenafil on cerebral hemodynamics in older adults

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## Abstract

**Objective:** Phosphodiesterase-5 inhibitors (PDE5Is) enhance vasodilation. We investigated the effects of PDE5I on cerebral hemodynamics during cognitive tasks using functional near-infrared spectroscopy (fNIRS). **Methods:** This study used a crossover design. Twelve cognitively healthy men participants (mean age,  $59 \pm 3$  years; range, 55–65 years) were recruited and randomly assigned to the experimental or control arm, then the experimental and control arm were exchanged after 1 week. Udenafil 100 mg was administered to participants in the experimental arm once daily for 3 days. We measured the fNIRS signal during the resting state and four cognitive tasks three times for each participant: at baseline, in the experimental arm, and in the control arm. **Results:** Behavioral data did not show a significant difference between the experimental and control arms. The fNIRS signal showed significant decreases in the experimental arm compared to the control arm during several cognitive tests: verbal fluency test (left dorsolateral prefrontal cortex,  $T = -3.02$ ,  $p = 0.014$ ; left frontopolar cortex,  $T = -4.37$ ,  $p = 0.002$ ; right dorsolateral prefrontal cortex,  $T = -2.59$ ,  $p = 0.027$ ), Korean-color word Stroop test (left orbitofrontal cortex,  $T = -3.61$ ,  $p = 0.009$ ), and social event memory test (left dorsolateral prefrontal cortex,  $T = -2.35$ ,  $p = 0.043$ ; left frontopolar cortex,  $T = -3.35$ ,  $p = 0.01$ ). **Interpretation:** Our results showed a paradoxical effect of udenafil on cerebral hemodynamics in older adults. This contradicts our hypothesis, but it suggests that fNIRS is sensitive to changes in cerebral hemodynamics in response to PDE5Is.

## Introduction

Phosphodiesterase 5 inhibitors (PDE5Is) are widely used to treat erectile dysfunction, but they are also approved for the treatment of pulmonary hypertension.<sup>1–4</sup> These drugs cause vasodilation by reducing cyclic guanosine monophosphate (cGMP) degradation in small blood vessels.<sup>5</sup> Consequently, researchers have raised questions about whether PDE5Is cause vasodilation of the cerebral blood vessels. If the effect on cerebral hemodynamics is proven to be significant, the use of PDE5Is could be a potential treatment strategy for cerebral small vessel disease. However, many previous studies failed to show the effect of PDE5Is on cerebral hemodynamics using various techniques, including

transcranial Doppler (TCD),<sup>6</sup> single-photon emission computed tomography (SPECT),<sup>7</sup> positron emission tomography (PET),<sup>8</sup> functional magnetic resonance imaging (fMRI),<sup>9</sup> and arterial spin labeling.<sup>10</sup> While these techniques were appropriate for measuring cerebral hemodynamics in large vessels, their ability to measure cerebral hemodynamics in small vessels is limited.

Functional near-infrared spectroscopy (fNIRS) measures cerebral tissue oxygenation, reflecting hemodynamic changes, which indicate brain activity. This has several benefits for measuring the response of cognitive stimulation including its non-invasiveness, portability, minimal motion artifacts, high temporal resolution, range and diversity of the participants, and lower costs compared to other

neuroimaging modalities such as fMRI, TCD, and SPECT.<sup>11,12</sup> The increase in oxygenated hemoglobin (HbO) identified using fNIRS was verified positively correlated with the blood oxygenation level-dependent (BOLD) response measured through fMRI.<sup>13</sup> A recent study also revealed the signal specificity of fNIRS, which detected increased blood oxygen saturation, whereas TCD did not detect any significant changes in the same participant.<sup>14</sup> Additionally, with its good tolerance to motion artifacts and portable nature, multichannel fNIRS is appropriate for monitoring reactive hemodynamic changes in brain regions in various settings, including cognitive tasks,<sup>15,16</sup> real-world situations,<sup>12,17–19</sup> and clinical populations.<sup>20,21</sup>

Cerebral blood flow (CBF) in response to neural activity is well known as neurovascular coupling.<sup>22</sup> In general, cognitive tasks related to neural activity in specific brain regions result in their increased blood oxygenation. Many studies have used cognitive tasks that engages complex cognitive processes, such as working memory, attention, and language processing. One study monitored prefrontal cortex activation during a working memory task using fNIRS.<sup>23</sup> Another study demonstrated the robustness of using fNIRS signals to measure and classify mental workload during resting and a task that needs attention processing.<sup>24</sup> Additionally, fNIRS was used in a study to evaluate age-related changes in cognitive functions by measuring cortical hemodynamic responses during a verbal fluency task (VFT).<sup>25</sup> Previous studies suggested that the benefits of fNIRS include measuring reactive hemodynamic changes during cognitive tasks, monitoring changes over time, and detecting changes associated with specific cognitive processes or clinical conditions. Thus, our research question is as follows: *Does udenafil affect cerebral hemodynamics in response to cognitive burden?* To the best of our knowledge, this is the first study to investigate whether PDE5Is affect fNIRS signal in response to cognitive tasks.

This is a pilot study to investigate the effects of udenafil—a PDE5I—on cerebral hemodynamics in participants with normal cognition. We hypothesized that udenafil would improve cerebral hemodynamics during cognitive burden. A crossover design was used to compare changes of behavioral scores and fNIRS signal during four cognitive tasks with and without the administration of udenafil. A multichannel fNIRS system was used to measure the regional changes of cerebral hemodynamics in response to cognitive burden in real-time.

## Methods

### Participant recruitment

As this is a pilot study to explore the effect of udenafil on cerebral hemodynamics, a sample size of 12 was

determined using information from previous studies as a guide.<sup>26,27</sup> Twelve healthy volunteers with no cognitive impairment were recruited between December 2020 and September 2021. All participants met the following criteria: (1) age between 55 and 65 years, (2) scores of the Korean version of the Mini-Mental State Examination (MMSE) within one standard deviation of those of the age- and education-matched mean, and (3) normal brain MRI. The exclusion criteria for healthy participants were: (1) stroke, myocardial infarction, or CABG surgery within 6 months; (2) cardiac failure, unstable angina, or life-threatening arrhythmia within 6 months; (3) poorly controlled diabetes mellitus and/or proliferative diabetic retinopathy; (4) spinal cord injury, radical prostatectomy, or radical pelvic surgery; (5) hypotension below 90/50 mmHg or poorly controlled hypertension over 170/100 mmHg; (6) renal or hepatic abnormalities; (7) retinitis pigmentosa; (8) active peptic ulceration within 1 year; (9) hematological disorders (sickle cell anemia, multiple myeloma, or leukemia) or bleeding disorders that could be the probable cause of priapism; (10) psychiatric disorder, drug abuse, or conditions that would influence the accuracy of our study; (11) ongoing treatment with nitrate or nitric oxide donor (e.g., nitroglycerin, isosorbide mononitrate, isosorbide dinitrate, amyl nitrate/nitrite, and sodium nitroprusside); (12) ongoing cancer chemotherapy; (13) ongoing treatment with anticoagulants; (14) ongoing ingestion of medication or food that influences metabolism of CYP3A4 in liver; (15) ongoing treatment with androgens (e.g., testosterone) or anti-androgens; (16) ongoing treatment with other PDE5 inhibitors (Viagra<sup>®</sup>, Levitra<sup>®</sup>, or Cialis<sup>®</sup>) or other erectile dysfunction (ED) regimens for ED treatment 2 weeks before the beginning of this study; (17) history of allergy to other PDE5 inhibitors (Viagra<sup>®</sup>, Levitra<sup>®</sup>, or Cialis<sup>®</sup>); (18) currently enrolled in another clinical trial or use of any investigational drug or device within 30 days; and (19) to prevent individuals with coronavirus disease-2019 (COVID-19) from participating, we conducted telephone interviews and instructed those with any suspicious symptoms to visit a nearby screening center for a free PCR test to ensure that participants with COVID-19 were identified and excluded.

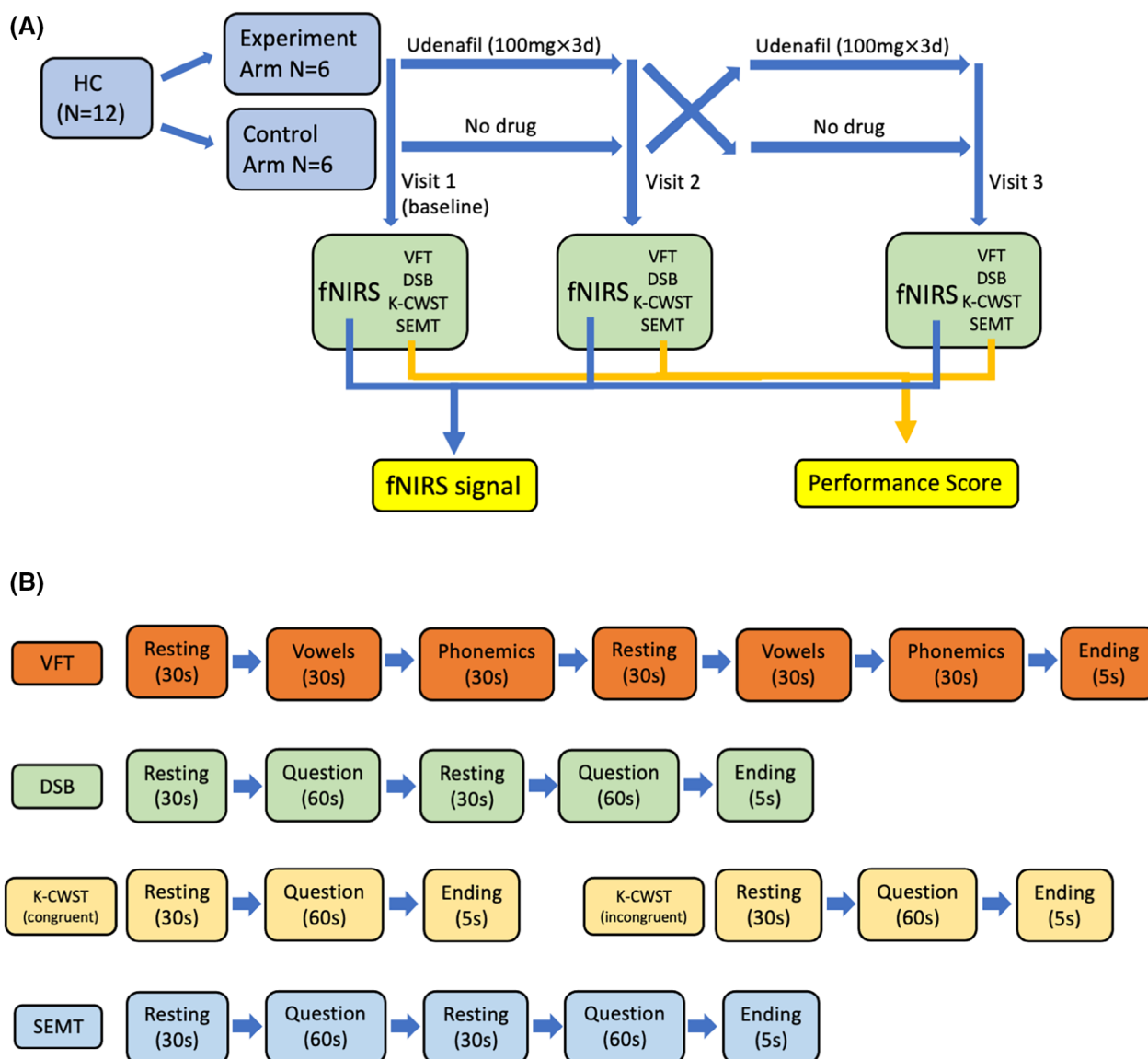
This study was approved by the Institutional Review Board of Jeonbuk National University Hospital (2019-12-026-002) and the Korean Ministry of Food and Drug Safety. This trial was registered with the Clinical Research Information Service, Republic of Korea ([cris.nih.go.kr](https://cris.nih.go.kr); KCT0005833) on 27 January 2021. All participants provided written informed consent after the study objectives were clearly explained. All methods were carried out in accordance with the approved guidelines.

## Experimental design

A crossover design was used in this study (Fig. 1). Cognitively healthy participants were randomly assigned to the experimental or control arm, and the experimental and control arms were exchanged after 1 week (block randomization: the block size was six). Udenafil 100 mg was administered to participants in the experimental arm once daily for 3 days. The time of taking the medication was 8 AM, and the test time was 10 AM. All participants performed the same four cognitive tasks three

times: at baseline, in the experimental arm, and in the control arm.

We measured fNIRS signals during cognitive tasks. To ensure that the same brain region was captured, we aligned the marking point on the front of the device to the center (between the eyes). To minimize the possible influence of lamplight or sunlight entering the device during data collection, we measured the fNIRS signals in a room with indirect lightning. To reduce the influence of the participant's hair on the measurements, we finger-combed their hair away from the forehead before the device was put on.



**Figure 1.** Experimental design. (A) A crossover design was employed. The participants were randomly assigned to the experimental and control arm, then the experimental and control arm were exchanged after 1 week. (B) Schematic diagram of cognitive task: VFT, DSB, K-CWST, and SEMT. DSB, digit span backward test; fNIRS, functional near-infrared spectroscopy; HC, healthy control; K-CWST, Korean-Color Word Stroop Test; SEMT, social event memory test; VFT, verbal fluency test.

After the position of the device was fixed from the front, the strap and Velcro hooks on the back were fixed to avoid moving the device during the experiment.

Participants were asked to sit on a chair and maintain a comfortable posture using a head-mounted fNIRS system. Before performing the cognitive tasks, participants were instructed to stare at the cross pattern on the screen for 3 min to obtain the resting state data. Participants performed four cognitive tasks using a tablet computer.

## Cognitive tasks

The experiment comprised four tasks: (1) VFT, (2) digit span backward test (DSB), (3) Korean-Color Word Stroop Test (K-CWST), and (4) social event memory test (SEMT). Thirty seconds of rest was provided between each task (Fig. 1).

### VFT

Participants were instructed to generate as many words as possible from the following phonemic categories: “s” and “g.” The procedure for each phonemic category consisted of three blocks: a 30-s initial rest, a 30-s pre-task, and a 30-s VFT task. During the rest period, participants were instructed to keep their eyes fixated on a cross mark at the center of the tablet screen. During the pre-task, participants were instructed to repeat aloud the five Korean vowels: “a,” “ae,” “i,” “o,” and “u.” During the VFT task, participants were instructed to produce as many Korean words as possible beginning with a designated phonemic category, “s” or “g.” The performance on VFT indicated the level of maintenance or updating, selection, and manipulation, as well as quantity of vocabulary and lexical access speed, reflecting language and frontal function.<sup>28</sup>

### DSB

Participants were instructed to respond with digits in the reverse order to that presented on the tablet screen. Digits were presented at a rate of one per second. Subsequently, a numeric pad was displayed on the tablet screen, and participants had to recall the sequence by tapping the pad in the reverse order. The “done” button had to be tapped once after the answer was completed. In total, three-digit span backward (DSB3) and four-digit span backward (DSB4) questions were presented. This task reflected participants’ verbal short-term and working memory.<sup>29–31</sup>

### K-CWST

We modified the K-CWST to accept responses by touching the tablet computer screen. Colored words were

shown in different colors than the meaning of the word on the tablet screen (e.g., the word “yellow” is presented in red text). In the congruent conditions, participants were instructed to respond by tapping the same word as the word itself. In the incongruent condition, participants were instructed to respond by tapping the name of the color of the word. The K-CWST reflects frontal-executive function.<sup>32,33</sup>

### SEMT

Before starting this task, participants were instructed to remember the conversation in the task as much as possible. Then, participants were required to watch a 5-min video clip and answer the question about the video clip on the tablet. There were six options for each question, and participants were asked to make a choice and input their answers into the tablet computer. The SEMT was designed to evaluate verbal, visual, and associative memory.<sup>34</sup>

## Experimental apparatus

A noninvasive, wearable, head-mounted fNIRS system (NIRSIT; OBELAB Inc., Seoul, Korea) was used in this study. Oxyhemoglobin (HbO<sub>2</sub>) values were obtained from the prefrontal region of the human brain using the fNIRS system. There were 24 laser sources (780/850 nm; maximum power under 1 mW) and 32 photodetectors with variable source-detector spacing (1.5, 2.12, 3.0, and 3.35 cm) in the fNIRS system, generating 204 measurement points at a sampling rate of 8.138 Hz. The oxygen saturation in the blood of the prefrontal cortex within the range of 15–95% (within 5 s of updated time) during the experiment was saved to the wirelessly connected tablet.

### fNIRS data analysis

For analysis purposes, 48 channels were created by a 3.0 cm separation between the sources and detectors in the prefrontal cortex. Hemodynamic changes were extracted using the Modified Beer–Lambert law in each channel. The data were filtered using high- and low-pass filters at 0.005 and 0.1 Hz, respectively, to eliminate cardiovascular artifacts and environmental noise and baseline corrected with the last 5 s of the pre-task as baseline for each block. We rejected poor-quality channels with a signal-to-noise ratio of less than 30 dB before extraction of hemodynamic data to prevent misinterpretation. Because block-designed tasks were performed in our study, we used task-locked block-averaging methods;<sup>35</sup> fNIRS data were recorded synchronously to the stimuli presentation and timeline of the tasks. The block-averaged hemodynamic response is

computed by averaging the HbO<sub>2</sub> and deoxyhemoglobine (HbR) across the task blocks. Hemodynamic responses of the baseline, and experimental and control arms were obtained from each participant. To compare the experimental and control arms after baseline correction, the average amplitude at baseline was subtracted from the average amplitude of the experimental and control arms.

$\Delta Control$  = mean amplitude of the control arm – mean amplitude of baseline.

$\Delta Udenafil$  = mean amplitude of the experimental arm – mean amplitude of baseline.

We then calculated the average value for eight subregions: the left and right dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), frontopolar cortex (FPC), and ventromedial prefrontal cortex. The channel numbers for each subregion were as follows: right DLPFC (1–3, 5, 6, 11, 17, 18), left DLPFC (19, 20, 33–35, 38, 39, 43), right FPC (7, 8, 12, 13, 21, 22, 25, 26), left FPC (23, 24, 27, 28, 36, 37, 41, 42), right orbitofrontal cortex (OFC) (14–16, 29, 30), and left OFC (31, 32, 46, 47, 48) (Fig. S1). We defined outliers as data points more than 1.5 interquartile range below the first quartile (Q1) and above the third quartile (Q3) and removed them.

## Statistical analysis

To compare the behavioral performance among the baseline, and experimental and control arms, we used the Friedman test because the variables did not follow a normal distribution. Dunn-Bonferroni posthoc tests were performed for multiple comparisons and corrections.

To compare the fNIRS signal, the mean values of eight regions of interest, the left and right DLPFC, FPC, OFC, and VLPFC, were calculated and then compared between  $\Delta Control$  and  $\Delta Udenafil$  using a paired t-test.

Statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) version 27.0 (IBM Corp. Armonk, NY). Statistical significance was set at  $p < 0.05$ .

## Results

### Demographics

We recruited 12 cognitively healthy men participants with a mean age of  $59 \pm 3$  years, mean years of education of  $16 \pm 3$  years, and mean K-MMSE score of  $30 \pm 1$ . One (8%) of the participants had hypertension, and none had diabetes. The mean International Prostate Symptom Score was  $9 \pm 6$  and the mean International Index of Erectile Function-5 score was  $35 \pm 22$  (Table 1).

**Table 1.** Demographics and clinical characteristics.

	Participants
Number of cases, <i>n</i>	12
Age, mean $\pm$ SD	$59 \pm 3$
Male sex, <i>n</i> (%)	12 (100%)
Education, mean $\pm$ SD	$16 \pm 3$
Hypertension	1 (8%)
Diabetes	0 (0%)
K-MMSE, mean $\pm$ SD	$30 \pm 1$
IPSS, mean $\pm$ SD	$9 \pm 6$
IIEF-5, mean $\pm$ SD	$35 \pm 22$

IIEF-5, International Index of Erectile Function-5; IPSS, International Prostate Symptom Score-5; MMSE, Mini-Mental State Examination; SD, standard deviation.

### Comparison of behavioral data

All participants performed four cognitive tasks (VFT, DSB, K-CWST, and SEMT), and their baseline, and experimental and control arm performances were compared (Table 2). The baseline indicated the performance scores of all participants in their baseline state, the experimental arm indicated the performance scores of all participants after 3 days of udenafil administration, and the control arm indicated the performance scores of all participants in their control state without udenafil.

Number of responses, accuracy, and response times were compared. Number of responses indicated the number of responses, regardless of correct or incorrect answers, whereas accuracy indicated the number of correct answers divided by the number of responses. Response time indicated the duration between the moment each question began to the moment the participants responded.

In the VFT, we compared only number of responses. Number of responses was not significantly different among the baseline and experimental and control arms.

In DSB3, the response time was significantly different among the three arms ( $p = 0.020$ ). Dunn-Bonferroni posthoc tests found that participants had shorter response times in the experimental arm than in the baseline ( $p = 0.022$ ). There were no significant differences between the control arm and baseline or experimental arms. The number of responses and accuracy showed no significant differences among the baseline and two arms. In DSB4, there were no significant differences in the number of responses, accuracy, or response time among the baseline and two arms.

In the K-CWST congruent task, there were significant differences in number of responses ( $p = 0.020$ ) and response time ( $p = 0.010$ ) among the baseline and two arms, and the posthoc test showed that number of

**Table 2.** Behavioral performance.

Test	Baseline median (IQR)	Control arm median (IQR)	Udenafil arm median (IQR)	p-value			
				Friedman	C vs. B	U vs. B	U vs. C
VFT							
Number of response	17.00 (14.75, 19.25)	18.00 (14.00, 19.00)	17.50 (13.75, 19.25)	0.976	1.000	1.000	1.000
DSB3							
Number of response	6.00 (6.00, 7.00)	7.00 (6.50, 7.00)	7.00 (5.75, 7.00)	0.206	0.723	1.000	0.497
Accuracy	1.00 (0.86, 1.00)	1.00 (1.00, 1.00)	0.94 (0.85, 1.00)	0.072	1.000	0.602	0.329
Response time (msec)	4309.47 (3844.04, 5305.82)	3717.71 (3222.79, 4014.71)	3735.57 (3219.40, 4079.25)	0.020	0.133	0.022	1.000
DSB4							
Number of response	4.50 (3.00, 6.00)	5.00 (3.50, 6.00)	6.00 (4.50, 7.00)	0.058	1.000	0.128	0.723
Accuracy	0.84 (0.56, 1.00)	0.83 (0.66, 0.86)	1.00 (0.81, 1.00)	0.191	1.000	0.602	0.602
Response time (msec)	5804.67 (4593.33, 7014.10)	5075.07 (4207.45, 5232.33)	4894.40 (3943.50, 5998.58)	0.121	0.178	0.297	1.000
K-CWST Congruent							
Number of response	40.00 (33.75, 41.00)	40.50 (36.00, 43.25)	42.00 (36.50, 43.00)	0.020	0.124	0.032	1.000
Accuracy	1.00 (0.98, 1.00)	1.00 (0.98, 1.00)	1.00 (0.98, 1.00)	0.733	1.000	1.000	1.000
Response time (msec)	1020.64 (975.81, 1293.96)	1012.23 (898.87, 1183.36)	942.79 (902.57, 1170.70)	0.010	0.157	0.009	0.922
K-CWST Incongruent							
Number of response	34.00 (29.00, 36.75)	36.50 (32.50, 38.75)	36.50 (33.00, 41.25)	0.002	0.043	0.002	1.000
Accuracy	0.97 (0.94, 1.00)	0.99 (0.97, 1.00)	1.00 (0.98, 1.00)	0.041	0.124	0.307	1.000
Response time (msec)	1285.29 (1156.22, 1601.49)	1146.99 (1050.93, 1400.94)	1171.79 (960.95, 1342.89)	0.003	0.096	0.002	0.662
SEMT							
Number of response	16.00 (15.00, 19.00)	22.50 (20.00, 28.25)	22.50 (20.75, 25.00)	0.001	0.002	0.007	1.000
Accuracy	0.78 (0.67, 0.84)	0.89 (0.83, 0.90)	0.88 (0.86, 0.91)	0.016	0.057	0.032	1.000
Response time (msec)	6328.92 (5926.54, 7448.70)	4959.69 (3936.05, 5933.10)	4898.56 (4418.65, 5474.06)	0.000	0.001	0.005	1.000

DSB, digit span backward test; K-CWST, Korean color word Stroop test; SEMT, social event memory test; VFT, verbal fluency test.

responses was significantly increased in the experimental arm compared to that in the baseline ( $p=0.032$ ); response time was significantly decreased in the experimental arm compared to that in the baseline ( $p=0.009$ ). There was no significant change in accuracy. In the K-CWST incongruent task, there were significant differences in number of responses ( $p=0.002$ ), accuracy ( $p=0.041$ ), and response time ( $p=0.003$ ) among the baseline and two arms. The posthoc test showed that number of responses was significantly increased in the experimental arm ( $p=0.002$ ) and control arm ( $p=0.043$ ) compared to the baseline. There was no significant difference in accuracy after Dunn-Bonferroni correction. Response time was significantly decreased in the experimental arm compared to that in the baseline ( $p=0.002$ ).

In the SEMT, there were significant differences in number of responses ( $p=0.001$ ), accuracy ( $p=0.016$ ), and response time ( $p<0.001$ ) among the three arms. The posthoc test showed that number of responses was significantly increased in the experimental ( $p=0.007$ ) and control arms ( $p=0.002$ ) compared to that in the baseline; accuracy was significantly increased in the experimental arm compared to that in the baseline ( $p=0.032$ ); and the response time was significantly decreased in the experimental ( $p=0.005$ ) and control arms ( $p=0.001$ ) compared to that in the baseline.

There was a tendency for scores to increase in the first, second, and third order in several tasks, regardless of whether they were included in the experimental or control arm (Fig. S2). In DSB3, a tendency of learning effect

was observed in response time; however, no learning effect was observed in number of responses in DSB3 and DSB4 and in response time in DSB4. In the SEMT task, a learning effect was observed in number of responses ( $p < 0.01$ ) and response time ( $p < 0.01$ ). No learning effect was observed in the VFT and K-CWST tasks. A crossover design was used to reduce the learning effect.

### Comparison of fNIRS data

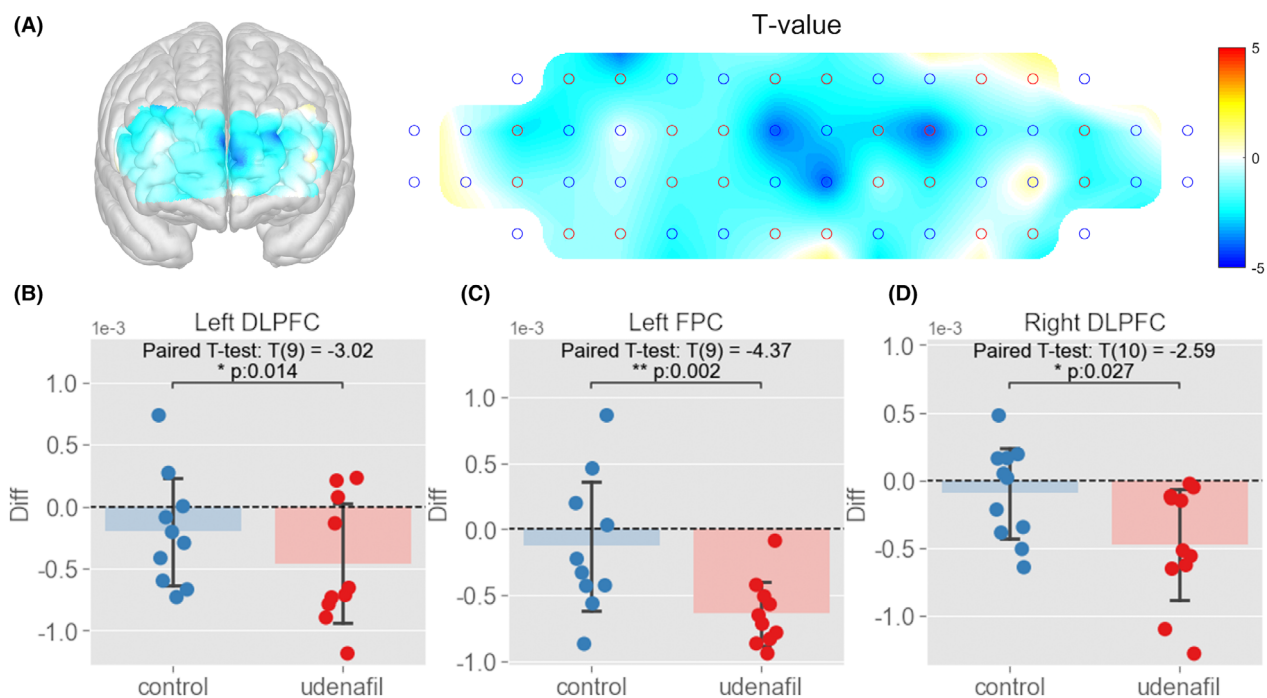
As for the comparison of  $\Delta Control$  and  $\Delta Udenafil$  in the four cognitive tasks,  $\Delta Udenafil$  decreased compared to  $\Delta Control$  in the prefrontal cortex during several cognitive tasks. In the VFT,  $\Delta Udenafil$  was significantly decreased compared to  $\Delta Control$  in the left DLPFC ( $T = -3.02$ ,  $p = 0.014$ ), left FPC ( $T = -4.37$ ,  $p = 0.002$ ), and right DLPFC ( $T = -2.59$ ,  $p = 0.027$ ) (Fig. 2, Fig. S3). In the DSB, there was no significant difference between  $\Delta Control$  and  $\Delta Udenafil$  in the DSB3 and DSB4 tasks (Fig. S4). In the K-CWST congruent task, no significant difference between  $\Delta Control$  and  $\Delta Udenafil$  was observed (Fig. S4). In the K-CWST incongruent task,  $\Delta Udenafil$  was significantly decreased in the left OFC ( $T = -3.61$ ,  $p = 0.009$ ), whereas  $\Delta Udenafil$  was significantly increased in the right OFC ( $T = 2.50$ ,  $p = 0.032$ ) and the right FPC ( $T = 2.61$ ,

$p = 0.028$ ) compared to  $\Delta Control$  (Fig. 3, Fig. S5). In the SEMT,  $\Delta Udenafil$  was significantly decreased in the left DLPFC ( $T = -2.35$ ,  $p = 0.043$ ) and left FPC ( $T = -3.35$ ,  $p = 0.010$ ) compared to  $\Delta Control$  (Fig. 4, Fig. S6). The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

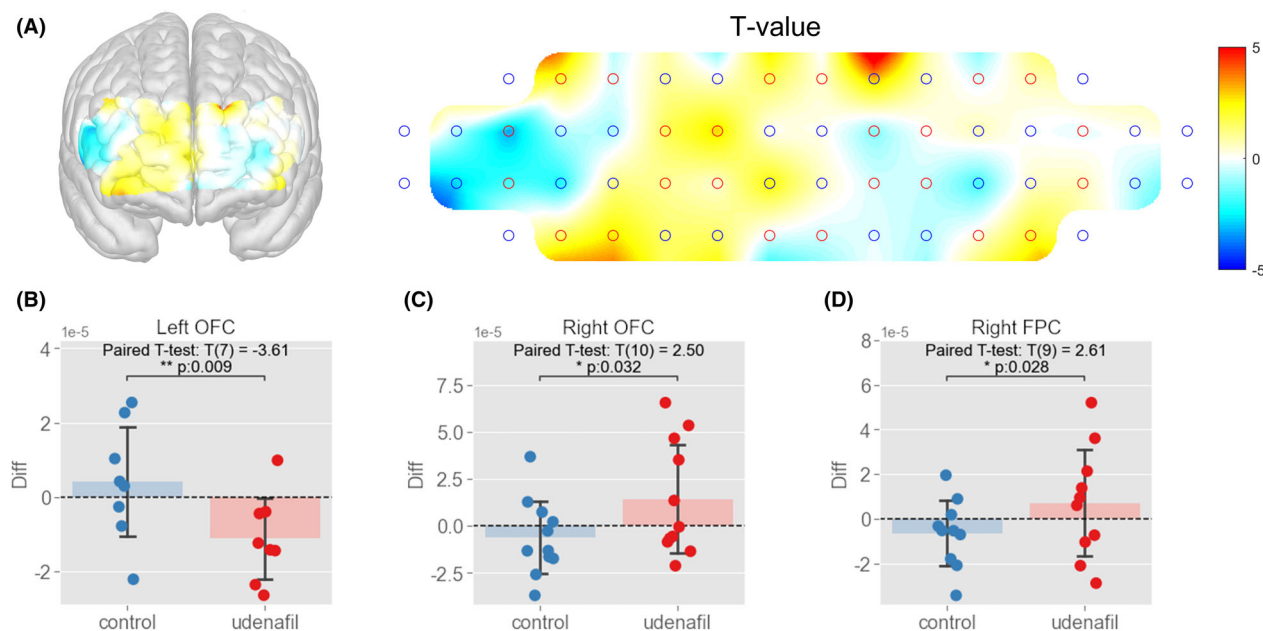
### Discussion

In this study, we explored the effects of PDE5I on cerebral hemodynamics in response to cognitive burden in cognitively healthy participants. In terms of behavioral data, the experimental and control arms showed better performance than the baseline in several tasks; however, there was no significant difference between the experimental and control arms. In contrast, in terms of fNIRS data,  $\Delta Udenafil$  decreased compared to  $\Delta Control$  in the prefrontal cortex during several cognitive tasks. Our results suggest that cerebral hemodynamics measured by fNIRS might be sensitive in detecting the effects of PDE5I.

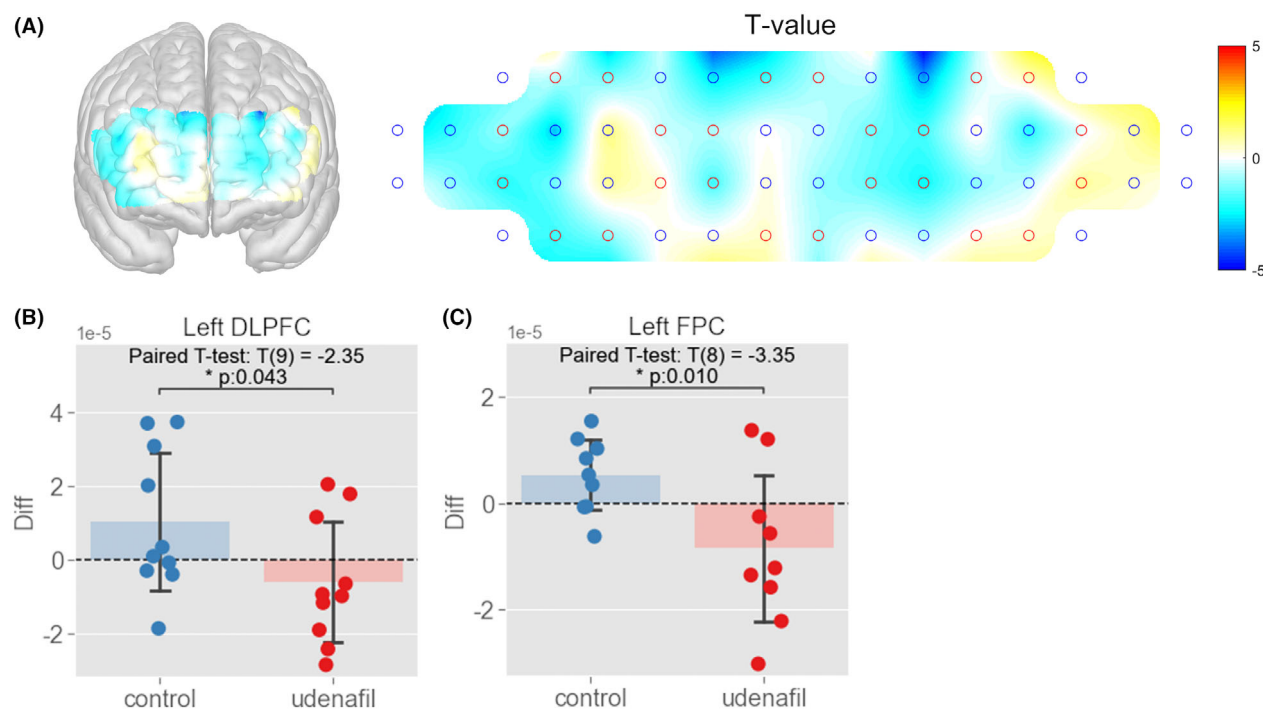
We measured cerebral oxygenation reflecting hemodynamic changes during the cognitive tasks using an fNIRS device. Previous studies have suggested that reactive CBF, which is the cerebrovascular response to demands such as



**Figure 2.** Comparison of fNIRS data in the verbal fluency test (VFT). (A)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left DLPFC, left FPC, and right DLPFC. (B)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left DLPFC ( $T = -3.02$ ,  $p = 0.014$ ). (C)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left FPC ( $T = -4.37$ ,  $p = 0.002$ ). (D)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the right DLPFC ( $T = -2.59$ ,  $p = 0.027$ ). DLPFC, dorsolateral prefrontal cortex; FPC, frontopolar cortex; fNIRS, functional near-infrared spectroscopy.



**Figure 3.** Comparison of fNIRS data in the Korean-Color Word Stroop Test (K-CWST) (Incongruent). (A)  $\Delta Udenafil$  was decreased in the left OFC whereas  $\Delta Udenafil$  was increased in the right OFC and right FPC, compared to  $\Delta Control$  in K-CWST incongruent task. (B) In K-CWST incongruent,  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left OFC ( $T = -3.61$ ,  $p = 0.009$ ). (C) In K-CWST incongruent,  $\Delta Udenafil$  was increased compared to  $\Delta Control$  in the right OFC ( $T = 2.50$ ,  $p = 0.032$ ). (D) In K-CWST incongruent,  $\Delta Udenafil$  was increased compared to  $\Delta Control$  in the right FPC ( $T = 2.61$ ,  $p = 0.028$ ). FPC, frontopolar cortex; fNIRS, functional near-infrared spectroscopy; OFC, orbitofrontal cortex.



**Figure 4.** Social Event Memory Test (SEMT). (A)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left DLPFC and left FPC. (B)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left DLPFC ( $T = -2.35$ ,  $p = 0.043$ ). (C)  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the left FPC ( $T = -3.35$ ,  $p = 0.010$ ). DLPFC, dorsolateral prefrontal cortex; FPC, frontopolar cortex.

hypercapnia and cognitive burden, but not resting CBF, was improved by a PDE5I.<sup>36</sup> Based on previous studies, we designed this study to measure cerebral hemodynamics during various cognitive tasks that stimulate working memory, association memory, and frontal-executive function.

Behavioral data indicated that the experimental and control arms showed better performance than the baseline in several tasks, indicating a learning effect. However, there was no significant difference between the experimental and control arms for any task. This result suggests that the administration of udenafil for 3 days was insufficient to affect cognitive task scores. Better performance in both the experimental and control arms compared to the baseline was probably related to learning effects. In our study, baseline tasks were always performed first, whereas the experimental and control tasks were performed second or third. In fact, there was a tendency for scores to increase in the first, second, and third order in several tasks, regardless of whether they were included in the experimental or control arm. To mitigate the learning effect, we averaged the data of visits 2 and 3 for each experimental and control arm.

Interestingly, fNIRS data showed that  $\Delta Udenafil$  was decreased compared to  $\Delta Control$  in the prefrontal cortex during cognitive tasks, except for DSB. We hypothesized that PDE5I would improve cerebral hemodynamics during cognitive burden and predicted that this improvement would be an increase in cerebral oxygenation; however, the actual results were consistent with a decrease in cerebral oxygenation. These results can be interpreted in several ways. First, a paradoxical decrease in cerebral oxygenation may be related to healthy participants in this study. Previous studies have found that cerebrovascular reactivity (CVR) is increased by PDE5I in patients with impaired responses to endothelial vasodilation.<sup>10,37–40</sup> However, a study showed that in healthy controls, CVR did not increase, but rather paradoxically decreased due to PDE5I, which is consistent with our results.<sup>40</sup> In addition, we recruited relatively young participants (age  $59 \pm 3$  years), which might have influenced the results. A study revealed a trend for greater CBF change by PDE5I with increasing age in patients with SVCI at least 65 years of age.<sup>41</sup> PDE5I-mediated effects on cerebral hemodynamics may not be evident in healthy participants aged <65 years. Alternatively, a paradoxical decrease in reactive CBF in the prefrontal cortex might be a response to an increase in CBF in deep brain structures. Previous studies have found increased CBF in the hippocampus in an animal model<sup>42</sup> and medial temporal lobe in patients with Alzheimer's disease<sup>43</sup> by sildenafil, a PDE5i. A recent study found a trend of increased CBF within the WMH due to tadalafil, another PDE5I, in patients with SVCI.<sup>43</sup> Because

our fNIRS device (NIRSIT; OBELAB Inc., Seoul, Korea) could only measure superficial cerebral oxygenation in the prefrontal cortex, we might not find an increase in other areas such as the medial temporal region, including the hippocampus or subcortical white matter.

Our study has several limitations. First, the small sample size and only 3-day drug administration period might have affected our results. Second, we measured only the superficial cerebral oxygenation in the prefrontal cortex and not that of deep structures, including the hippocampus or white matter. Third, we could not perform the breath-holding task to measure CVR. Although our study aimed to measure changes of cerebral hemodynamics in response to cognitive burden, a comparison with the breath-holding task reflecting CVR would have been beneficial. Therefore, in future studies with larger sample sizes, both healthy controls and patients with SVCI by age should be recruited to study the PDE5I-mediated effects of participant factors. In addition, a device that can detect changes in both superficial and deep brain structures must be considered. Despite these limitations, the strength of our study is that cerebral hemodynamics was measured during cognitive tasks. Our study suggests the possibility of using cerebral hemodynamics measured with the fNIRS device as a biomarker of PDE5I-mediated effects.

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## Conflict of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Author Contributions

Duk L. Na and Ko Woon Kim contributed to the study conception and design. Qi Wang and Ko Woon Kim performed statistical analysis and wrote the first draft of the manuscript. Byoung-Soo Shin, Sun-Young Oh, and Yu Seob Shin organized the database and wrote sections of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.**